Search Strategy

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     FILE 'MEDLINE' ENTERED AT 16:26:29 ON 09 AUG 2001

                  E LOWELL G H/AU
L1             34 S E3
                  E VANCOTT T C/AU
L2             44 S E3
L3             42 S L2 NOT L1
                  E BIRX D L/AU
L4             82 S E3
L5             56 S L4 NOT (L1 OR L2)

     FILE 'USPATFULL' ENTERED AT 17:06:02 ON 09 AUG 2001

                  E LOWELL GEORGE H/IN
L6              4 S E3
                  E VANCOTT THOMAS C/IN
                  E BIRX DEBORAH L/IN
L7              2 S E3 OR E4

     FILE 'MEDLINE' ENTERED AT 17:11:50 ON 09 AUG 2001

L8            114 S PROTEOSOM?
L9              6 S L8 AND (MUCOSAL)
L10             1 S L9 NOT L1
L11            25 S L8 AND VACCIN?
L12            10 S L11 NOT L1
                  E REDFIELD R R/AU
L13            98 S E3
L14            11 S L13 AND PY=1991
                  E DESAI S M/AU
L15            52 S E3
L16             1 S L15 AND 451
L17             6 S OLIGOMERIC GP160 OR OLIGO-GP160 OR O-GP160
L18        116939 S VACCIN?
L19            11 S L18 AND (HYDROPHOBIC ANCHOR OR HYDROPHOBIC FOOT)
                  E MANNINO R J/AU
L20            22 S E3
                  E YANG C/AU
L21           353 S E3
L22            20 S L21 AND PY=1995
L23             1 S L22 AND (HIV OR SIV)
L24            39 S L18 AND (LAUROYL OR LAUROYL-CYS OR LAUROYL-CYSTEINE OR PALMIT

     FILE 'USPATFULL' ENTERED AT 18:30:03 ON 09 AUG 2001

L25           114 S PROTEOSOM?
L26            69 S L25 AND VACCIN?
L27             5 S L26 AND (LAUROYL OR LAUROYL-CYSTEINE OR PALMITOYL OR PALMITOY
L28            16 S L26 AND (HIV OR SIV)
L29             5 S L28 AND GP160

     FILE 'WPIDS' ENTERED AT 18:35:22 ON 09 AUG 2001

                  E LOWELL G H/IN
L30             5 S E3
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		E	VANCOTT T C/IN
L31	1	S	E3
		E	BIRX D L/IN
L32	28	S	E3 OR E2
L33	49	S	PROTEOSOM?
L34	18	S	L33 AND VACCIN?
L35	13	S	L34 AND (HIV OR SIV)
L36	12	S	L35 NOT L30

L1 ANSWER 22 OF 34 MEDLINE
88154753 Document Number: 88154753. PubMed ID: 3346624. Peptides bound to proteosomes via hydrophobic feet become highly immunogenic without adjuvants. ***Lowell G H*** ; Smith L F; Seid R C; Zollinger W D. (Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC 20307.) JOURNAL OF EXPERIMENTAL MEDICINE, (1988 Feb 1) 167 (2) 658-63. Journal code: I2V; 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Addition of either a lauroyl or a pentapeptide (FLLAV) hydrophobic foot to the NH2 terminus of a small, synthetic peptide allowed the peptide to hydrophobically complex to meningococcal outer membrane protein proteosomes by simple dialysis. Both conventional and LPS-hyporesponsive mice immunized with these complexes without any adjuvants developed high-titered and persistent anti-peptide IgG. Since proteosomes have been safely given to many people and since important antigenic determinants are generally hydrophilic, this system should be widely applicable to the development of peptide vaccines for human use.

L1 ANSWER 20 OF 34 MEDLINE
88204920 Document Number: 88204920. PubMed ID: 2452484. Proteosome-lipopeptide vaccines: enhancement of immunogenicity for malaria CS peptides. ***Lowell G H*** ; Ballou W R; Smith L F; Wirtz R A; Zollinger W D; Hockmeyer W T. (Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100.) SCIENCE, (1988 May 6) 240 (4853) 800-2. Journal code: UJ7; 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Proteosomes are hydrophobic, membranous, multimolecular preparations of meningococcal outer membrane proteins that are also B cell mitogens. These characteristics suggested that proteosomes may serve as carrier proteins and adjuvants to enhance peptide immunogenicity. Although high titers of malaria circumsporozoite (CS) antibodies protect against malaria, vaccines thus far tested in humans have been insufficiently immunogenic to be clinically useful. Here it is shown that synthetic CS peptides hydrophobically complexed to proteosomes by way of lauroyl-cysteine become highly immunogenic in mice without other adjuvants. The high titers of antibodies produced and the safety of proteosomes in humans suggest that this novel system is widely applicable for the development of peptide vaccines to protect against many diseases.

L1 ANSWER 18 OF 34 MEDLINE
90162869 Document Number: 90162869. PubMed ID: 2696152. Proteosome-hydrophobic 'foot' malaria peptide vaccines for Plasmodium falciparum and P. vivax. ***Lowell G H*** ; Ballou W R; Smith L F; Zollinger W D; Hockmeyer W T. (Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100.) TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, (1989) 83 Suppl 101-2. Journal code: WBU; 7506129. ISSN: 0035-9203. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The immunogenicity of synthetic peptides representing the repeating portions of circumsporozoite proteins of Plasmodium sporozoites was greatly increased by complexing them to proteosomes via hydrophobic moieties added to their amino termini. Proteosomes have been used safely in people in the development of meningococcal vaccines and therefore proteosome-peptide vaccines are prime candidates for use against malaria.

L1 ANSWER 16 OF 34 MEDLINE
91108074 Document Number: 91108074. PubMed ID: 2125617. Preparation of

proteosome-based vaccines. Correlation of immunogenicity with physical characteristics. Ruegg C L; Jaffe R I; Koster B; Sadoff J C; Zollinger W D; ***Lowell G H*** ; Strand M. (Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) JOURNAL OF IMMUNOLOGICAL METHODS, (1990 Dec 31) 135 (1-2) 101-9. Journal code: IFE; 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB In order to facilitate the use of proteosome-based vaccines, we have identified and analyzed the parameters that affect their immunogenicity. As a model system we used synthetic peptides (LCF6) containing sequences from the immunodominant (NANP)n tandem repeat region of the P. falciparum circumsporozoite protein, hydrophobically complexed to multimeric protein preparations (proteosomes) of meningococcal outer membrane proteins (OMP), since we have previously shown that high levels of anti-(NANP)n IgG can be elicited in mice by use of this novel adjuvant system (Lowell et al., 1988a). We have now examined these preparations by velocity sedimentation and measured their ability to elicit an IgG response in mice. Velocity sedimentation of freshly mixed OMP and LCF6, without dialysis, produced a limited number of small complexes, whereas dialysis of the mixture for 4 d yielded heterogeneously sized complexes that became more homogeneous when the dialysis was carried out for 7 or 10 days. The most homogeneous of these peptide-proteosome complexes (those dialyzed for 10 days) induced substantial levels of anti-(NANP)n IgG in mice, and shorter periods of dialysis resulted in vaccines that induced proportionately lower titers. Analysis of a series of preparations with varying LCF6: OMP ratios (w/w) showed that the degree of peptide substitution of the proteosomes was inversely proportional to the rate of sedimentation of the complexes and that there exists an optimal degree of lipopeptide complexing to the proteosomes. Our results suggest that the parameters affecting the immunogenicity of the peptide-proteosome complexes are: (i) hapten density, and (ii) size of the complex. Furthermore, sedimentation analysis of peptide-proteosome immunogens may serve as a rapidly performed assay of immunogenic potency.

L1 ANSWER 14 OF 34 MEDLINE
93056536 Document Number: 93056536. PubMed ID: 1431131. Induction of protective immunity in mice using a 62-kDa recombinant fragment of a Schistosoma mansoni surface antigen. Soisson L M; Masterson C P; Tom T D; McNally M T; ***Lowell G H*** ; Strand M. (Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) JOURNAL OF IMMUNOLOGY, (1992 Dec 1) 149 (11) 3612-20. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Mice exposed to radiation-attenuated cercariae of Schistosoma mansoni are highly resistant to challenge infection, and sera from these mice can confer partial resistance when transferred to naive recipients. These sera recognize Ag present in schistosomular and adult worms, among them an Ag of 200 kDa. A cDNA encoding a 62-kDa portion of this Ag was cloned; the deduced amino acid sequence of this cDNA clone shares homology with myosins of other species. To assess the immunoprophylactic potential, we carried out vaccination trials in mice using the recombinant polypeptide expressed as a fusion protein with beta-galactosidase presented in the form of proteosome complexes with the outer membrane protein of meningococcus. The level of protection achieved was 32%, and this level could be increased to 75% by removal of those amino acids included in the fusion protein that were derived from the vector to yield a polypeptide, designated rIrV-5. A similar level of protection was achieved when mice were immunized with the same dose of rIrV-5 in the form of protein complexes but without outer membrane protein, suggesting that protection

did not require the use of adjuvant. However, at least three immunizations were necessary to achieve protection. Using mAb and sera from mice vaccinated with rIrV-5, we demonstrated that the native protein recognized by antibodies against rIrV-5 is a 200-kDa protein that is expressed on the surface of newly transformed schistosomula. The protection achieved with rIrV-5 in mice encourages additional studies of its potential as a vaccine candidate for the prevention of schistosomiasis.

L1 ANSWER 13 OF 34 MEDLINE
93273486 Document Number: 93273486. PubMed ID: 8500877. Immunogenicity and efficacy of oral or intranasal Shigella flexneri 2a and Shigella sonnei proteosome-lipopolysaccharide vaccines in animal models. Orr N; Robin G; Cohen D; Arnon R; ***Lowell G H*** . (Medical Corps, Israel Defence Force, Rehovot.) INFECTION AND IMMUNITY, (1993 Jun) 61 (6) 2390-5. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Immunity against shigellosis has been shown to correlate with the presence of antibodies specific for Shigella lipopolysaccharide (LPS). We here propose a new candidate vaccine for shigellosis composed of purified Shigella flexneri 2a or Shigella sonnei LPS hydrophobically complexed with group C type 2b Neisseria meningitidis outer membrane protein proteosomes. Immunization of mice either orally or intranasally with this complex induced specific homologous anti-LPS antibodies in both intestinal and respiratory secretions as well as in sera. Strong anamnestic responses were found after two or three immunizations. LPS alone, alkaline-detoxified LPS, or alkaline-detoxified LPS complexed with proteosomes was not effective. Oral or intranasal immunization of guinea pigs with two or more doses of this proteosome-LPS vaccine elicited homologous protection against Shigella keratoconjunctivitis (Sereny test). These data demonstrate that proteosomes can be used as an effective mucosal vaccine delivery system and that orally or intranasally administered acellular vaccines can protect against Shigella infections.

L1 ANSWER 11 OF 34 MEDLINE
95012749 Document Number: 95012749. PubMed ID: 7927807. Enhancement of anti-Shigella lipopolysaccharide (LPS) response by addition of the cholera toxin B subunit to oral and intranasal proteosome-Shigella flexneri 2a LPS vaccines. Orr N; Arnon R; Rubin G; Cohen D; Bercovier H; ***Lowell G*** *** H*** . (Medical Corps, Israel Defence Force, Military Post 02149, Rehovot, Israel.) INFECTION AND IMMUNITY, (1994 Nov) 62 (11) 5198-200. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Addition of the cholera toxin B subunit to oral and intranasal proteosome-Shigella flexneri 2a lipopolysaccharide vaccines improved their immunogenicities. Enhancement of anti-O-Shigella immunoglobulin A levels was most evident in lung lavages following oral immunization and in lung and intestinal fluids when suboptimal doses were used with either

L1 ANSWER 10 OF 34 MEDLINE
95286295 Document Number: 95286295. PubMed ID: 7768627. Intransal or intragastric immunization with proteosome-Shigella lipopolysaccharide vaccines protects against lethal pneumonia in a murine model of Shigella infection. Mallett C P; Hale T L; Kaminski R W; Larsen T; Orr N; Cohen D; ***Lowell G H*** . (Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, D.C. 20307, USA.) INFECTION AND IMMUNITY, (1995 Jun) 63 (6) 2382-6. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Mice immunized intransally or intragastrically with proteosome vaccines

containing either Shigella sonnei or S. flexneri 2a lipopolysaccharide were protected against lethal pneumonia caused by homologous organisms in an experimental murine intranasal challenge model of Shigella infection. Histopathological analysis demonstrated that immunization also protected against the progressive lesions resulting from invasion of the pulmonary mucosa by S. sonnei. These data show that mucosal proteosome-lipopolysaccharide vaccines can protect against lethal bacterial pneumonia and indicate that such vaccines are promising candidates for protection against intestinal shigellosis.

L1 ANSWER 8 OF 34 MEDLINE
96155146 Document Number: 96155146. PubMed ID: 8585293. Intranasal immunization of mice against influenza with synthetic peptides anchored to proteosomes. Levi R; Aboud-Pirak E; Leclerc C; ***Lowell G H*** ; Arnon R. (Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.) VACCINE, (1995 Oct) 13 (14) 1353-9. Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Synthetic vaccines that are based on peptides representing immunogenic epitopes require a carrier molecule as well as an adjuvant in order to be effective. The choice of carriers or adjuvants approved for use in humans is very limited, and a considerable effort is devoted to develop new and efficient delivery systems. One of these vehicles utilizes preparations of outer membranes of meningococci, that form hydrophobic interactions, denoted proteosomes. Immunogenic proteins and peptides can be anchored to these proteosomes vesicles, which may serve as both carrier and adjuvant functions. In the present study we examined the ability of proteosomes to present epitopes of influenza, to elicit specific anti-influenza responses and to protect mice against viral challenge after intranasal immunization. Three influenza peptides were used--one corresponding to amino acid residues 91-108 of the haemagglutinin surface glycoprotein of H3 subtype, which comprises a B-cell epitope, and two from the internal nucleoprotein--a T-helper cell (Th) epitope (residues 55-69) and a cytotoxic T-lymphocyte (CTL) epitope (147-158). Mice were immunized intranasally (i.n.) with preparations containing each of the above epitopes, or various combinations thereof. The results obtained with this system demonstrate that influenza epitopes represented by synthetic peptides anchored to a proteosome carrier elicit both humoral and cellular specific immune responses, that can lead to partial protection of the mice from viral challenge. The importance of immunizing with vaccines containing both B- and T-cell peptide epitopes was emphasized by the demonstration that such vaccines elicited longer lasting immunity and led to more effective protection against influenza viral challenge.

L1 ANSWER 7 OF 34 MEDLINE
96201582 Document Number: 96201582. PubMed ID: 8613381. Intranasal and intramuscular proteosome-staphylococcal enterotoxin B (SEB) toxoid vaccines: immunogenicity and efficacy against lethal SEB intoxication in mice. ***Lowell G H*** ; Kaminski R W; Grate S; Hunt R E; Charney C; Zimmer S; Colleton C. (Division of Pathology, Walter Reed Army Institute of Research, Washington, DC 20307, USA.) INFECTION AND IMMUNITY, (1996 May) 64 (5) 1706-13. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Intranasal or intramuscular (i.m.) immunization of mice and i.m. immunization of rabbits with formalinized staphylococcal enterotoxin B (SEB) toxoid in saline elicited higher anti-SEB serum immunoglobulin G (IgG) titers when the toxoid was formulated with proteosomes. In addition, intranasal immunization of mice with this proteosome-toxoid vaccine elicited high levels of anti-SEB IgA in lung and intestinal secretions,

whereas the toxoid without proteosomes did not. Two i.m. immunizations with proteosome-toxoid plus alum also induced higher murine serum responses than alum-adjuvanted toxoid without proteosomes. Furthermore, proteosome-toxoid delivered intranasally in saline or i.m. with either saline or alum afforded significant protection against lethal SEB challenge in two D-galactosamine-sensitized murine models of SEB intoxication, i.e., the previously described i.m. challenge model and a new respiratory challenge model of mucosal SEB exposure. Efficacy correlated with the induction of high serum levels of anti-SEB IgG. In contrast, intranasal or i.m. immunization with toxoid in saline without proteosomes was not significantly protective in either challenge model. Proteosome-toxoid plus alum given i.m. also elicited more significant protection against respiratory challenge than the alum-adjuvanted toxoid alone. The capacity of proteosomes to enhance both i.m. and intranasal immunogenicity and efficacy of SEB toxoid indicates that testing such proteosome-SEB toxoid vaccines in the nonhuman primate aerosol challenge model of SEB intoxication prior to immunogenicity trials in humans is warranted. These data expand the applicability of the proteosome mucosal vaccine delivery system to protein toxoids and suggest that respiratory delivery of proteosome vaccines may be practical for enhancement of both mucosal and systemic immunity against toxic or infectious diseases.

L1 ANSWER 6 OF 34 MEDLINE
97000551 Document Number: 97000551. PubMed ID: 8843636. Effective immunization of mice against cutaneous leishmaniasis using an intrinsically adjuvanted synthetic lipopeptide vaccine. Frankenburg S; Axelrod O; Kutner S; Greenblatt C L; Klaus S N; Pirak E A; McMaster R; ***Lowell G H*** . (Department of Dermatology, Hadassah Medical Organization, Jerusalem, Israel.) VACCINE, (1996 Jun) 14 (9) 923-9. Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Two peptides representing predicted T-cell epitopes of gp63, a major surface glycoprotein of the parasite Leishmania major, were used in vaccines tested in a murine model of cutaneous leishmaniasis. Either subcutaneous or intraperitoneal immunization in saline with a peptide representing gp63 amino acids 467-482 (p467) significantly protected CBA mice against the development of severe cutaneous lesions only when the peptide was intrinsically adjuvanted by covalently adding a lauryl-cysteine moiety (LC-p467) to its amino terminus during synthesis. In marked contrast, administration of p467 alone, cysteinyl-p467 or gp63 protein in saline resulted in some disease exacerbation. Splenic cells of LC-p467 immunized mice stimulated in vitro with LC-p467 displayed strong proliferative responses and secretion of IL-2, IFN-tau and GM-CSF (but not IL-4 and IL-10) suggesting that immunization with the lipopeptide induced the TH1 type cytokine responses associated with cell-mediated immunity. The safety, efficacy, ease of production and standardization of such lipopeptide vaccines suggest that they have significant potential for the development of vaccines for humans against leishmaniasis or other parasitic or viral diseases that require cell-mediated immunity for protection.

L1 ANSWER 5 OF 34 MEDLINE
97045161 Document Number: 97045161. PubMed ID: 8890226. Immunogenicity and efficacy against lethal aerosol staphylococcal enterotoxin B challenge in monkeys by intramuscular and respiratory delivery of proteosome-toxoid vaccines. ***Lowell G H*** ; Colleton C; Frost D; Kaminski R W; Hughes M; Hatch J; Hooper C; Estep J; Pitt L; Topper M; Hunt R E; Baker W; Baze W B. (Division of Pathology, Walter Reed Army Institute of Research, Washington, D.C. 20307, USA.) INFECTION AND IMMUNITY, (1996 Nov) 64 (11) 4686-93. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country:

United States. Language: English.

AB Staphylococcal enterotoxin B (SEB), a primary cause of food poisoning, is also a superantigen that can cause toxic shock after traumatic or surgical staphylococcal wound [correction of would] infections or viral influenza-associated staphylococcal superinfections or when aerosolized for use as a potential biologic warfare threat agent. Intranasal or intramuscular (i.m.) immunization with formalinized SEB toxoid formulated with meningococcal outer membrane protein proteosomes has previously been shown to be immunogenic and protective against lethal respiratory or parenteral SEB challenge in murine models of SEB intoxication. Here, it is demonstrated that immunization of nonhuman primates with the proteosome-SEB toxoid vaccine is safe, immunogenic, and protective against lethal aerosol challenge with 15 50% lethal doses of SEB. Monkeys (10 per group) were primed i.m. and given booster injections by either the i.m. or intratracheal route without adverse side effects. Anamnestic anti-SEB serum immunoglobulin G (IgG) responses were elicited in all monkeys, but strong IgA responses in sera and bronchial secretions were elicited both pre- and post-SEB challenge only in monkeys given booster injections intratracheally. The proteosome-SEB toxoid vaccine was efficacious by both routes in protecting 100% of monkeys against severe symptomatology and death from aerosolized-SEB intoxication. These data confirm the safety, immunogenicity, and efficacy in monkeys of parenteral and respiratory vaccination with the proteosome-SEB toxoid, thereby supporting clinical trials of this vaccine in humans. The safety and enhancement of both bronchial and systemic IgA and IgG responses by the proteosome vaccine delivered by a respiratory route are also encouraging for the development of mucosally delivered proteosome vaccines to protect against SEB and other toxic or infectious respiratory pathogens.

L1 ANSWER 4 OF 34 MEDLINE
97347310 Document Number: 97347310. PubMed ID: 9203649. Proteosomes, emulsomes, and cholera toxin B improve nasal immunogenicity of human immunodeficiency virus gp160 in mice: induction of serum, intestinal, vaginal, and lung IgA and IgG. ***Lowell G H*** ; Kaminski R W; VanCott T C; Slike B; Kersey K; Zawoznik E; Loomis-Price L; Smith G; Redfield R R; Amselem S; Birx D L. (Division of Pathology, Walter Reed Army Institute of Research, Washington, DC, USA.) JOURNAL OF INFECTIOUS DISEASES, (1997 Feb) 175 (2) 292-301. Journal code: IH3; 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Intranasal immunization of mice with human immunodeficiency virus (HIV) rgp160 complexed to proteosomes improved anti-gp160 serum IgA and IgG titers, increased the number of gp160 peptides recognized, and stimulated anti-gp160 intestinal IgA compared with immunization with uncomplexed rgp160 in saline. These enhanced responses were especially evident when either a bioadhesive nanoemulsion (emulsomes) or cholera toxin B subunit (CTB) was added to the proteosome-rgp160 vaccine. Furthermore, anti-gp160 IgG and IgA in vaginal secretions and fecal extracts were induced after intranasal immunization with proteosome-rgp160 delivered either in saline or with emulsomes. Formulation of uncomplexed rgp160 with emulsomes or CTB also enhanced serum and selected mucosal IgA responses. Induction of serum, vaginal, bronchial, intestinal, and fecal IgA and IgG by intranasal proteosome-rgp160 vaccines delivered in saline or with emulsomes or CTB is encouraging for mucosal vaccine development to help control the spread of HIV transmission and AIDS.

L1 ANSWER 3 OF 34 MEDLINE
1998129366 Document Number: 98129366. PubMed ID: 9469464. HIV-1 neutralizing antibodies in the genital and respiratory tracts of mice intranasally immunized with oligomeric gp160. VanCott T C; Kaminski R W;

Mascola J R; Kalyanaraman V S; Wassef N M; Alving C R; Ulrich J T; ***Lowell G H*** ; Birx D L. (Henry M. Jackson Foundation, Walter Reed Army Institute of Research, Rockville, MD 20850, USA.. tvancott@hiv.hjf.org) . JOURNAL OF IMMUNOLOGY, (1998 Feb 15) 160 (4) 2000-12. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Because mucosal surfaces are a primary route of HIV-1 infection, we evaluated the mucosal immunogenicity of a candidate HIV-1 vaccine, oligomeric gp160 (o-gp160). In prior studies, parenteral immunization of rabbits with o-gp160 elicited broad neutralizing serum Ab responses against both T cell line-adapted HIV-1 and some primary HIV-1 isolates. In this study, nasal immunization of mice with o-gp160, formulated with liposomes containing monophosphoryl lipid A (MPL), MPL-AF, proteosomes, emulsomes, or proteosomes with emulsomes elicited strong gp160-specific IgG and IgA responses in serum as well as vaginal, lung, and intestinal washes and fecal pellets. The genital, respiratory, and intestinal Abs were determined to be locally produced. No mucosal immune responses were measurable when the immunogen was given s.c. Abs from sera and from vaginal and lung washes preferentially recognized native forms of monomeric gp120, suggesting no substantial loss in protein tertiary conformation after vaccine formulation and mucosal administration. Inhibition of HIV-1MN infection of H9 cells was found in sera from mice immunized intranasally with o-gp160 formulated with liposomes plus MPL, proteosomes, and proteosomes plus emulsomes. Formulations of o-gp160 with MPL-AF, proteosomes, emulsomes, or proteosomes plus emulsomes elicited HIV-1MN-neutralizing Ab in lung wash, and formulations with proteosomes, emulsomes, or proteosomes plus emulsomes elicited HIV-1MN-neutralizing Ab in vaginal wash. These data demonstrate the feasibility of inducing both systemic and mucosal HIV-1-neutralizing Ab by intranasal immunization with an oligomeric gp160 protein.

L1 ANSWER 1 OF 34 MEDLINE
2001334144 Document Number: 21295109. PubMed ID: 11401998. Safety and immunogenicity of a proteosome-Shigella flexneri 2a lipopolysaccharide vaccine administered intranasally to healthy adults. Fries L F; Montemarano A D; Mallett C P; Taylor D N; Hale T L; ***Lowell G H*** . (Intellivax, Inc., Baltimore, Maryland 21227, USA.. lfries@intellivax.com) . INFECTION AND IMMUNITY, (2001 Jul) 69 (7) 4545-53. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We studied the safety and immunogenicity of a Shigella flexneri 2a vaccine comprising native S. flexneri 2a lipopolysaccharide (LPS) complexed to meningococcal outer membrane proteins-proteosomes-in normal, healthy adults. A two-dose series of immunizations was given by intranasal spray, and doses of 0.1, 0.4, 1.0, and 1.5 mg (based on protein) were studied in a dose-escalating design. The vaccine was generally well tolerated. The most common reactions included rhinorrhea and nasal stuffiness, which were clearly dose related ($P < \text{or} = 0.05$). These reactions were self-limited and generally mild. The vaccine elicited S. flexneri 2a LPS-specific immunoglobulin A (IgA), IgG, and IgM antibody-secreting cells (ASCs) in a dose-responsive manner. At doses of 1.0 or 1.5 mg, highly significant ($P < 0.001$) increases in ASCs of all antibody isotypes occurred and 95% of subjects had an ASC response in at least one antibody isotype. Dose-related serum antibody responses were observed, with geometric mean two- to fivefold rises in specific serum IgA and IgG titers and two- to threefold rises in IgM in the 1.0- and 1.5-mg-dose groups ($P < 0.0001$ for each isotype). Elevated serum antibody levels persisted through day 70. Increases in fecal IgG and IgA and also in urinary IgA specific for S. flexneri 2a LPS were demonstrated. These were most consistent and approached statistical significance ($P = 0.02$ to 0.12 for various

measures) on day 70 after the first dose. The magnitude of immune responses to intranasally administered proteosome-S. flexneri 2a LPS vaccine is similar to those reported for live vaccine candidates associated with protective efficacy in human challenge models, and further evaluation of this product is warranted.

L3 ANSWER 25 OF 42 MEDLINE
97296236 Document Number: 97296236. PubMed ID: 9151820. Antibodies with specificity to native gp120 and neutralization activity against primary human immunodeficiency virus type 1 isolates elicited by immunization with oligomeric gp160. ***VanCott T C*** ; Mascola J R; Kaminski R W; Kalyanaraman V; Hallberg P L; Burnett P R; Ulrich J T; Rechtman D J; Birx D L. (Henry M. Jackson Foundation, Rockville, Maryland 20850, USA.. tvancott@hiv.hjf.org) . JOURNAL OF VIROLOGY, (1997 Jun) 71 (6) 4319-30. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Current human immunodeficiency virus type 1 (HIV-1) envelope vaccine candidates elicit high antibody binding titers with neutralizing activity against T-cell line-adapted but not primary HIV-1 isolates. Serum antibodies from these human vaccine recipients were also found to be preferentially directed to linear epitopes within gp120 that are poorly exposed on native gp120. Systemic immunization of rabbits with an affinity-purified oligomeric gp160 protein formulated with either Alhydrogel or monophosphoryl lipid A-containing adjuvants resulted in the induction of high-titered serum antibodies that preferentially bound epitopes exposed on native forms of gp120 and gp160, recognized a restricted number of linear epitopes, efficiently bound heterologous strains of monomeric gp120 and cell surface-expressed oligomeric gp120/gp41, and neutralized several strains of T-cell line-adapted HIV-1. Additionally, those immune sera with the highest oligomeric gp160 antibody binding titers had neutralizing activity against some primary HIV-1 isolates, using phytohemagglutinin-stimulated peripheral blood mononuclear cell targets. Induction of an antibody response preferentially reactive with natively folded gp120/gp160 was dependent on the tertiary structure of the HIV-1 envelope immunogen as well as its adjuvant formulation, route of administration, and number of immunizations administered. These studies demonstrate the capacity of a soluble HIV-1 envelope glycoprotein vaccine to elicit an antibody response capable of neutralizing primary HIV-1 isolates.

L3 ANSWER 36 OF 42 MEDLINE
95325634 Document Number: 95325634. PubMed ID: 7602128. Characterization of a soluble, oligomeric HIV-1 gp160 protein as a potential immunogen. ***VanCott T C*** ; Veit S C; Kalyanaraman V; Earl P; Birx D L. (Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, MD 20850, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (1995 Jun 14) 183 (1) 103-17. Journal code: IFE; 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB We have assessed the oligomeric structure and antigenic properties of an affinity purified gp160 protein (oligo-gp160) using biosensor technology. Sucrose gradient purification analysis identified the existence of tetrameric, dimeric and monomeric forms of the protein. Reactivity to a broad panel of monoclonal antibodies specific for oligomeric gp160, discontinuous epitopes within monomeric gp120 and several linear epitopes within gp120 (V3) and gp41 was demonstrated. International sera from several countries, where HIV-1 clades A-F are prevalent, including type O from Cameroon, were reactive with oligo-gp160 indicating conserved antigenic epitopes. Enhanced immunologic reactivity per gp160 molecule was obtained with oligo-gp160 as compared to other current HIV-1(IIIB) subunit

monomeric envelope gp120/gp160 immunogens suggesting higher HIV-1 envelope protein mimicry. HIV-1 antibodies from sera during acute HIV-1 infection were detectable by oligo-gp160 prior to detection with either a recombinant, monomeric gp120 protein or several commercial HIV-1 screening kits suggesting antibodies sensitive to oligomeric gp160 structure may be present earlier in infection. The oligomeric nature of this gp160 protein preparation and high reactivity with divergent mAbs and HIV-1 sera support the use of this protein as an HIV-1 immunogen.

L3 ANSWER 39 OF 42 MEDLINE
94267254 Document Number: 94267254. PubMed ID: 7515931. Dissociation rate of antibody-gp120 binding interactions is predictive of V3-mediated neutralization of HIV-1. ***VanCott T C*** ; Bethke F R; Polonis V R; Gorny M K; Zolla-Pazner S; Redfield R R; Birx D L. (Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, MD 20850.) JOURNAL OF IMMUNOLOGY, (1994 Jul 1) 153 (1) 449-59. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB mAbs specific for the V3 loop of HIV-1 are capable of neutralizing laboratory strains of HIV-1 in vitro. In this report we use surface plasmon resonance and biosensor technology to demonstrate that the ability of V3-specific mAbs to neutralize HIV-1(MN) correlated with the dissociation rate constant of the homologous mAb-gp120 binding interaction. mAbs capable of binding diverse strains of gp120 with similar association rate constants exhibited marked differences in the dissociation rate. The dissociation rate, and not the association rate, was found to be predictive of the neutralization capacity of the mAb. Furthermore, synthetic peptides corresponding to the V3 loop of HIV-1(IIIB, MN) yielded quantitatively similar binding kinetic profiles abrogating the need for purified recombinant gp120 protein and potentially facilitating the screening of more diverse isolates. Biosensor immobilized V3 peptides were found to mimic their conformational structure in solution. This offers advantages to peptides studied by ELISA where some degree of denaturation and masking of epitopes can occur upon adsorption of peptides to plastic surfaces. The impact of amino acid substitutions within epitopes on subsequent mAb binding was dissected by observing alterations in dissociation rates. The technique provides rapid kinetic analyses of V3 Ab binding interactions with diverse V3 sequences, facilitating the efficient screening and selection of those most likely to possess the broadest and most potent HIV-1 neutralizing potentials.

L12 ANSWER 7 OF 10 MEDLINE
94014422 Document Number: 94014422. PubMed ID: 8409436. Protective immunity in baboons ***vaccinated*** with a recombinant antigen or radiation-attenuated cercariae of Schistosoma mansoni is antibody-dependent. Soisson L A; Reid G D; Farah I O; Nyindo M; Strand M. (Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) JOURNAL OF IMMUNOLOGY, (1993 Nov 1) 151 (9) 4782-9. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Mice ***vaccinated*** with radiation-attenuated cercariae of Schistosoma mansoni exhibit high levels of resistance to challenge infection. We have previously shown that sera from these mice recognize polypeptides that are expressed on the surface of newly transformed schistosomula. We have cloned and sequenced a cDNA that encodes a 62-kDa portion of one of these polypeptides. ***Vaccination*** of mice with this 62-kDa polypeptide (designated rlrV-5) elicits high antibody titers and significant resistance to challenge infection. We report here the

results of a ***vaccination*** trial in baboons with the rlrV-5 or radiation-attenuated cercariae. rlrV-5 was presented either in the form of protein micelles or complexed with the outer membrane protein of meningococcus to form ***proteosomes*** . The level of protection achieved in these groups ranged from 0 to 54%, with a mean of 27.7%. In baboons exposed to radiation-attenuated cercariae the level of protection was very high, with a mean of 84%. The resistance observed after ***vaccination*** with rlrV-5 or radiation-attenuated cercariae was reflected in the overall histopathology. ***Vaccination*** of baboons with rlrV-5 or radiation-attenuated cercariae elicited an antibody response against epitopes exposed on the surface of newly transformed schistosomula. In the case of baboons ***vaccinated*** with radiation-attenuated cercariae, this response was not limited to epitopes encompassed by rlrV-5. Analysis of individual baboon sera by ELISA demonstrated that there was a direct correlation between the anti-rlrV-5 titer and resistance to challenge worm burden, suggesting that the immunoprotective mechanism is antibody-dependent.

L14 ANSWER 6 OF 11 MEDLINE
91238907 Document Number: 91238907. PubMed ID: 1674589. A phase I evaluation of the safety and immunogenicity of vaccination with recombinant gp160 in patients with early human immunodeficiency virus infection. Military Medical Consortium for Applied Retroviral Research. ***Redfield R R*** ; Birx D L; Ketter N; Tramont E; Polonis V; Davis C; Brundage J F; Smith G; Johnson S; Fowler A; +. (Department of Retroviral Research, Walter Reed Army Institute of Research, Rockville, Md. 20850.) NEW ENGLAND JOURNAL OF MEDICINE, ***(1991 Jun 13)*** 324 (24) 1677-84. Journal code: NOW; 0255562. ISSN: 0028-4793. Pub. country: United States. Language: English.

AB BACKGROUND. Despite multiple antiviral humoral and cellular immune responses, infection with the human immunodeficiency virus (HIV) results in a progressively debilitating disease. We hypothesized that a more effective immune response could be generated by post-infection vaccination with HIV-specific antigens. METHODS. We performed a phase I trial of the safety and immunogenicity of a vaccine prepared from molecularly cloned envelope protein, gp160, in 30 volunteer subjects with HIV infection in Walter Reed stage 1 or 2. The vaccine was administered either on days 0, 30, and 120 or on days 0, 30, 60, 120, 150, and 180. HIV-specific humoral and cellular immune responses were measured; local and systemic reactions to vaccination, including general measures of immune function, were monitored. RESULTS. In 19 of the 30 subjects both humoral and cellular immunity to HIV envelope proteins increased in response to vaccination with gp160. Seroconversion to selected envelope epitopes was observed, as were new T-cell proliferative responses to gp160. Response was associated with the CD4 cell count determined before vaccination (13 of 16 subjects [81 percent] with greater than 600 cells per milliliter responded, as compared with 6 of 14 [43 percent] with less than or equal to 600 cells per milliliter; P = 0.07) and with the number of injections administered (87 percent of subjects randomly assigned to receive six injections responded, as compared with 40 percent of those assigned to three injections; P = 0.02). Local reactions at the site of injection were mild. There were no adverse systemic reactions, including diminution of general in vitro or in vivo cellular immune function. After 10 months of follow-up, the mean CD4 count had not decreased in the 19 subjects who responded, but it had decreased by 7.3 percent in the 11 who did not respond. CONCLUSIONS. This gp160 vaccine is safe and immunogenic in volunteer patients with early HIV infection. Although it is too early to know whether this approach will be clinically useful, further scientific and therapeutic evaluation of HIV-specific vaccine therapy is warranted. Similar vaccines may prove to be effective for other chronic infections.

L16 ANSWER 1 OF 1 MEDLINE
87041461 Document Number: 87041461. PubMed ID: 3490666. Molecular cloning and primary nucleotide sequence analysis of a distinct human immunodeficiency virus isolate reveal significant divergence in its genomic sequences. ***Desai S M*** ; Kalyanaraman V S; Casey J M; Srinivasan A; Andersen P R; Devare S G. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1986 Nov) 83 (21) 8380-4. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In an effort to evaluate data on genomic relatedness among the various human immunodeficiency viruses (HIVs), we have molecularly cloned a virus isolate designated HIV (CDC- ***451***). Preliminary characterization of the HIV (CDC- ***451***) clone indicated that the restriction enzyme map was distinct from those of other known HIV isolates. Analysis of the primary nucleotide sequence of the regions encoding the structural proteins and comparison with sequences known for other HIV isolates indicated substantial differences for HIV (CDC- ***451***). The sequences encoding the group-specific antigen gene, although they showed some variation, were conserved to a greater extent than were those encoding envelope proteins. In the envelope gene sequences, most of the changes (up to 24.5% divergence) were located in the amino-terminal region encoding a glycoprotein with a Mr of 120,000. The carboxyl-terminal region, encoding a protein of Mr 41,000, was more highly conserved. The variation in the sequences encoding envelope proteins may have important implications for the antigenic properties and/or pathogenicity of the disease and for its detection and ultimate eradication.

L17 ANSWER 4 OF 6 MEDLINE
97296236 Document Number: 97296236. PubMed ID: 9151820. Antibodies with specificity to native gp120 and neutralization activity against primary human immunodeficiency virus type 1 isolates elicited by immunization with ***oligomeric*** ***gp160*** . VanCott T C; Mascola J R; Kaminski R W; Kalyanaraman V; Hallberg P L; Burnett P R; Ulrich J T; Rechtman D J; Birx D L. (Henry M. Jackson Foundation, Rockville, Maryland 20850, USA.. tvancott@hiv.hjf.org) . JOURNAL OF VIROLOGY, (1997 Jun) 71 (6) 4319-30. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Current human immunodeficiency virus type 1 (HIV-1) envelope vaccine candidates elicit high antibody binding titers with neutralizing activity against T-cell line-adapted but not primary HIV-1 isolates. Serum antibodies from these human vaccine recipients were also found to be preferentially directed to linear epitopes within gp120 that are poorly exposed on native gp120. Systemic immunization of rabbits with an affinity-purified ***oligomeric*** ***gp160*** protein formulated with either Alhydrogel or monophosphoryl lipid A-containing adjuvants resulted in the induction of high-titered serum antibodies that preferentially bound epitopes exposed on native forms of gp120 and gp160, recognized a restricted number of linear epitopes, efficiently bound heterologous strains of monomeric gp120 and cell surface-expressed oligomeric gp120/gp41, and neutralized several strains of T-cell line-adapted HIV-1. Additionally, those immune sera with the highest ***oligomeric*** ***gp160*** antibody binding titers had neutralizing activity against some primary HIV-1 isolates, using phytohemagglutinin-stimulated peripheral blood mononuclear cell targets. Induction of an antibody response preferentially reactive with natively folded gp120/gp160 was dependent on the tertiary structure of the HIV-1 envelope immunogen as well as its adjuvant formulation, route of administration, and number of immunizations administered. These studies

demonstrate the capacity of a soluble HIV-1 envelope glycoprotein vaccine to elicit an antibody response capable of neutralizing primary HIV-1 isolates.

L17 ANSWER 5 OF 6 MEDLINE
96211471 Document Number: 96211471. PubMed ID: 8648672. Folding, assembly, and intracellular trafficking of the human immunodeficiency virus type 1 envelope glycoprotein analyzed with monoclonal antibodies recognizing maturational intermediates. Otteken A; Earl P L; Moss B. (Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892-0455, USA.) JOURNAL OF VIROLOGY, (1996 Jun) 70 (6) 3407-15. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Monoclonal antibodies (MAbs) that bind linear or conformational epitopes on monomeric or oligomeric human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins were screened for their recognition of maturational intermediates. On the basis of reactivities with gp160 at different times after pulse-labeling, the MAbs were sorted into groups that exhibited binding which was immediate and constant, immediate but transient, delayed, late, or very late. This grouping was consistent with the selectivity of the MAbs for structural features of gp160. Thus, a MAb to the V3 loop reacted with envelope proteins at all times, in accord with the relative conformational independence and accessibility of the epitope. Several MAbs that preferentially react with monomeric gp160 exhibited diminished binding after the pulse. A 10-min tag occurred before gp160 reacted with conformational MAbs that inhibited CD4 binding. The availability of epitopes for other conformational MAbs, including some that react equally with monomeric and ***oligomeric*** ***gp160*** and some that react better with oligomeric forms, was half-maximal in 30 min and closely followed the kinetics of gp160 oligomerization. Remarkably, there was a 1- to 2-h delay before gp160 reacted with stringent oligomer-specific MAbs. After 4 h, approximately 20% of the gp160 was recognized by these MAbs. Epitopes recognized by monomerspecific or CD4-blocking MAbs but not by oligomer-dependent MAbs were present on gp160 molecules associated with the molecular chaperone BiP/GRP78. MAbs with a preference for monomers reacted with recombinant or HIV-1 envelope proteins in the endoplasmic reticulum, whereas the oligomer-specific MAbs recognized them in the Golgi complex. Additional information regarding gp160 maturation and intracellular trafficking was obtained by using brefeldin A, dithiothreitol, and a low temperature.

L17 ANSWER 6 OF 6 MEDLINE
95325634 Document Number: 95325634. PubMed ID: 7602128. Characterization of a soluble, oligomeric HIV-1 gp160 protein as a potential immunogen. VanCott T C; Veit S C; Kalyanaraman V; Earl P; Birx D L. (Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, MD 20850, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (1995 Jun 14) 183 (1) 103-17. Journal code: IFE; 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB We have assessed the oligomeric structure and antigenic properties of an affinity purified gp160 protein (***oligo*** - ***gp160***) using biosensor technology. Sucrose gradient purification analysis identified the existence of tetrameric, dimeric and monomeric forms of the protein. Reactivity to a broad panel of monoclonal antibodies specific for ***oligomeric*** ***gp160*** , discontinuous epitopes within monomeric gp120 and several linear epitopes within gp120 (V3) and gp41 was demonstrated. International sera from several countries, where HIV-1 clades A-F are prevalent, including type O from Cameroon, were reactive with ***oligo*** - ***gp160*** indicating conserved antigenic

epitopes. Enhanced immunologic reactivity per gp160 molecule was obtained with ***oligo*** - ***gp160*** as compared to other current HIV-1(IIIB) subunit monomeric envelope gp120/gp160 immunogens suggesting higher HIV-1 envelope protein mimicry. HIV-1 antibodies from sera during acute HIV-1 infection were detectable by ***oligo*** - ***gp160*** prior to detection with either a recombinant, monomeric gp120 protein or several commercial HIV-1 screening kits suggesting antibodies sensitive to ***oligomeric*** ***gp160*** structure may be present earlier in infection. The oligomeric nature of this gp160 protein preparation and high reactivity with divergent mAbs and HIV-1 sera support the use of this protein as an HIV-1 immunogen.

L19 ANSWER 5 OF 11 MEDLINE
96057691 Document Number: 96057691. PubMed ID: 7551224. Liposomal presentation of antigens for human ***vaccines*** . Gluck R. (Department of Virology, Swiss Serum and Vaccine Institute Bern, Switzerland.) PHARMACEUTICAL BIOTECHNOLOGY, (1995) 6 325-45. Ref: 142. Journal code: BYR; 9310302. ISSN: 1078-0467. Pub. country: United States. Language: English.

AB Liposomes are considered prime candidates to improve the immunogenicity of both antigens with ***hydrophobic*** ***anchor*** sequences and soluble, nonmembrane proteins or synthetic peptides. During the 20 years since liposomes were first demonstrated to have adjuvant potential, studies have shown that variation in liposomal size, lipid composition, surface charge, membrane fluidity, lipid-protein composition, anchor molecules, and fusogenicity can significantly influence results. In addition, antigen location (e.g., whether it is adsorbed or covalently coupled to the liposome surface or encapsulated in liposomal aqueous compartments) may also be important. Analysis of these variables as well as a comparison of the various techniques used to ensure the efficacy, stability, homogeneity, and safety of liposomal ***vaccine*** have been discussed.

L19 ANSWER 4 OF 11 MEDLINE
96057693 Document Number: 96057693. PubMed ID: 7551226. Lipid matrix-based ***vaccines*** for mucosal and systemic immunization. Mannino R J; Gould-Fogerite S. (Department of Laboratory Medicine and Pathology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark 07103, USA.) PHARMACEUTICAL BIOTECHNOLOGY, (1995) 6 363-87. Ref: 59. Journal code: BYR; 9310302. ISSN: 1078-0467. Pub. country: United States. Language: English.

AB For more than a decade our laboratories have been combining concepts in biochemistry, virology, and immunology in order to develop a conceptual basis for ***vaccine*** design. Our long-term goals have been to construct simple and well-defined immunogens that would stimulate specific immune responses in vivo. Using this approach, we hypothesized that it should be possible to define the structural and biochemical parameters of an immunogen that are necessary and sufficient to stimulate designated effector arms of the immune system. Through the use of covalently coupled peptide complexes, we have been able to define minimal requirements for the induction of humoral immune responses (Mannino et al., 1992). This represents a significant advance in eliciting an immune response to peptides, because it requires only peptides and phospholipids in the absence of additional adjuvants. It is different from the previous use of peptides and liposomes since here the peptides are covalently linked to a ***hydrophobic*** ***anchor*** and integrated into the phospholipid complex, rather than passively adsorbed or encapsulated. The presentation of peptide as part of a peptide-phospholipid complex (in contrast to encapsulation or nonspecific absorption) may be more similar to the

natural presentation of an epitope in the context of an in vivo antigenic challenge. This technology also allows us to incorporate B and Th epitopes in a number of forms--as a single peptide, as two peptides in the same liposome, or as a peptide with viral glycoproteins in the same liposome. These data also demonstrate that Th epitopes do not have to be covalently linked to the B-cell epitope in order to provide help for that epitope. The implications of the data reported here are significant for both basic science and applied technologies. In basic science, the peptide-phospholipid complexes are potentially useful for analyzing the cooperative effects of B- and T-cell epitopes in the in vivo immune response. Since the peptide-phospholipid complexes are totally synthetic and highly immunogenic, they may be constructed in any formulation required to answer questions on the roles of B and T cells in promoting an immune response. Furthermore, since the number of antigenic sites is limited only by the number of peptides included in the peptide-phospholipid complexes, these constructs may be useful in producing antisera or monoclonal antibodies to weakly antigenic regions of a large protein, since the lack of antigenic competition should enhance the immunogenicity of these regions. Clinically, this technology will expand the potential for subunit ***vaccines*** .(ABSTRACT TRUNCATED AT 400 WORDS)

L20 ANSWER 10 OF 22 MEDLINE
90302545 Document Number: 90302545. PubMed ID: 1694612. Defining minimal requirements for antibody production to peptide antigens. Goodman-Snitkoff G; Eisele L E; Heimer E P; Felix A M; Andersen T T; Fuerst T R; ***Mannino R J*** . (Department of Microbiology and Immunology, Albany Medical College, NY 12208.) VACCINE, (1990 Jun) 8 (3) 257-62. Journal code: X60; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The role that individual determinants play in modulating the immune response of an organism to a pathogen is often obscured because of the complexity of the pathogen. In order to gain a better appreciation of the role of individual determinants in the immune response, a pathogen may be dissociated into smaller components, for example peptides representing specific epitopes. These isolated components are often poorly immunogenic and historically have required the use of adjuvants to stimulate antibody production. This report defines the minimal essential requirements for antibody production to a peptide in this system. These are the ability to stimulate both B- and T-helper lymphocytes, anchorage in a phospholipid complex and multivalency within the complex. When these conditions are met, no additional adjuvants are necessary. This procedure has allowed us to identify three distinct T-helper cell epitopes from HIV gp160. In addition, this information has been used to produce a simple, totally synthetic and highly immunogenic preparation for the production of antibodies to peptides.

L20 ANSWER 5 OF 22 MEDLINE
95169526 Document Number: 95169526. PubMed ID: 7865342. Lipid matrix-based subunit vaccines: a structure-function approach to oral and parenteral immunization. Gould-Fogerite S; Edghill-Smith Y; Kheiri M; Wang Z; Das K; Feketeova E; Canki M; ***Mannino R J*** . (Department of Laboratory Medicine and Pathology, UMDNJ-New Jersey Medical School, Newark 07103.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1994) 10 Suppl 2 S99-103. Ref: 14. Journal code: ART; 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Immunization is today the most effective defense mechanism against microbial infections. Although highly effective vaccines are currently available for a number of infectious diseases, vaccine formulations can

still be improved in a number of important areas. The ability to induce antigen-specific humoral and cell-mediated immunity is crucial to the development of effective prophylactic and therapeutic vaccines for HIV and other pathogens. The approach of our laboratory has been to design and test simple, highly defined antigen-lipid complexes that would stimulate antibody and cell-mediated immune responses in the absence of any nonspecific immunological activators such as Freund's adjuvant, lipopolysaccharide (LPS), or alum. These studies have provided insight into the relationships between the properties of an immunogen and the induction of the desired immune responses. We have previously utilized this approach to define the minimal structures required for the induction of antibody responses. Our more recent studies have focused on defining the parameters involved in the induction of cell-mediated and mucosal immune responses. Toward this end we have developed a new type of subunit vaccine that is effective when given orally or intramuscularly, and elucidated structure-function relationships in peptide vaccines that affect induction of CD8+ cell responses.

L23 ANSWER 1 OF 1 MEDLINE
96003881 Document Number: 96003881. PubMed ID: 7568235. The human and simian immunodeficiency virus envelope glycoprotein transmembrane subunits are palmitoylated. ***Yang C*** ; Spies C P; Compans R W. (Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, ***(1995 Oct 10)*** 92 (21) 9871-5. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The envelope proteins of human immunodeficiency virus (***HIV***) and simian immunodeficiency virus (***SIV***) were found to be modified by fatty acylation of the transmembrane protein subunit gp41. The precursor gp160 was also palmitoylated prior to its cleavage into the gp120 and gp41 subunits. The palmitic acid label was sensitive to treatment with hydroxylamine or 2-mercaptoethanol, indicating that the linkage is through a thioester bond. Treatment with cycloheximide did not prevent the incorporation of [3H]palmitic acid into the ***HIV*** envelope protein, indicating that palmitoylation is a posttranslation modification. In contrast to other glycoproteins, which are palmitoylated at cysteine residues within or close to the membrane-spanning hydrophobic domain, the palmitoylation of the ***HIV*** -1 envelope proteins occurs on two cysteine residues, Cys-764 and Cys-837, which are 59 and 132 amino acids, respectively, from the proposed membrane-spanning domain of gp41. Sequence comparison revealed that one of these residues (Cys-764) is conserved in the cytoplasmic domains of almost all ***HIV*** -1 isolates and is located very close to an amphipathic region which has been postulated to bind to the plasma membrane.

L24 ANSWER 35 OF 39 MEDLINE
90066706 Document Number: 90066706. PubMed ID: 2586628. In vivo priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide ***vaccine*** . Deres K; Schild H; Wiesmuller K H; Jung G; Rammensee H G. (Institut fur Organische Chemie, Universitat Tubingen.) NATURE, (1989 Nov 30) 342 (6249) 561-4. Journal code: NSC; 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Cytotoxic T lymphocytes (CTL) constitute an essential part of the immune response against viral infections. Such CTL recognize peptides derived from viral proteins together with major histocompatibility complex (MHC) class I molecules on the surface of infected cells, and usually require in vivo priming with infectious virus. Here we report that synthetic viral peptides covalently linked to tripalmitoyl-S-glycerylcysteinyl-seryl-

serine (P3CSS) can efficiently prime influenza-virus-specific CTL in vivo. These lipopeptides are able to induce the same high-affinity CTL as does the infectious virus. Our data are not only relevant to ***vaccine*** development, but also have a bearing on basic immune processes leading to the transition of virgin T cells to activated effector cells in vivo, and to antigen presentation by MHC class I molecules.

L24 ANSWER 33 OF 39 MEDLINE
93013891 Document Number: 93013891. PubMed ID: 1398749. Palmitic acid conjugation of a protein antigen enhances major histocompatibility complex class II-restricted presentation to T cells. Robinson J H; Case M C; Brooks C G. (Department of Immunology, The Medical School, University of Newcastle upon Tyne, U.K.) IMMUNOLOGY, (1992 Aug) 76 (4) 593-8. Journal code: GH7; 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The effect on antigenicity of covalent attachment of lipid groups to a protein antigen was investigated. Coupling of palmitic acid to ovalbumin (OVA) enhanced major histocompatibility complex (MHC) class II-restricted presentation to most OVA-specific murine T-cell clones in vitro. The enhanced antigenicity of ***palmitoylated*** antigen was localized to the level of presentation of the synthetic peptide epitope, OVA 323-339. T-cell responses to ***palmitoylated*** antigen were more difficult to block with anti-MHC class II antibodies than responses to native antigen. However, T-cell proliferation to ***palmitoyl*** (p)-OVA and native (n)-OVA were blocked equally by anti-CD4 antibodies. Taken together, the results suggest that lipid conjugation of a protein antigen leads to the formation of a lipopeptide T-cell epitope with increased affinity of binding to MHC class II and/or T-cell receptor (TcR). These results have implications for the design of synthetic peptide ***vaccines*** .

L24 ANSWER 31 OF 39 MEDLINE
93122974 Document Number: 93122974. PubMed ID: 1282504. Solid phase peptide synthesis of lipopeptide ***vaccines*** eliciting epitope-specific B-, T-helper and T-killer cell response. Wiesmuller K H; Bessler W G; Jung G. (Institute of Organic Chemistry, University of Tubingen, Germany.) INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, (1992 Sep-Oct) 40 (3-4) 255-60. Ref: 55. Journal code: GSD; 0330420. ISSN: 0367-8377. Pub. country: Denmark. Language: English.

AB Lymphocyte subpopulations involved in the self and nonself recognition processes are antibody producing cells, T-helper cells and T-killer cells. By using lipopeptide adjuvants and lipopeptide-antigen conjugates each of the major pathways of immune response can be specifically addressed on the molecular level of minimized synthetic lipopeptide ***vaccines*** . The immunologically active principle of the lipopeptide constructs is the synthetic N-terminus of bacterial lipoprotein, tri- ***palmitoyl*** -S-glycerylcysteine, which can be covalently linked to B-, T-helper and CTL epitopes. Methods of multiple peptide synthesis based on Merrifield's solid-phase synthesis allow the economic production of the high numbers of overlapping lipopeptides required for the complete immunological screening of viral proteins.

L24 ANSWER 28 OF 39 MEDLINE
95021329 Document Number: 95021329. PubMed ID: 7935506. Lipophilic multiple antigen peptide system for peptide immunogen and synthetic ***vaccine*** . Huang W; Nardelli B; Tam J P. (Department of Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232-2363.) MOLECULAR IMMUNOLOGY, (1994 Oct) 31 (15) 1191-9. Journal code: NG1; 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We describe the development and structural requirements of a new lipophilic multiple antigen peptide (lipoMAP) system for immunogens that contains a built-in lipophilic adjuvant and has the ability to elicit cytotoxic T-lymphocytes (CTLs). In addition to the peptide antigens of choice at the amino terminus, the basic lipoMAP design consists of three components: a tetravalent symmetrical core matrix containing two levels of branching beta-alanyl-lysine as a building unit, a hydrophilic Ser-Ser dipeptide linker, and at the carboxyl terminus, ***palmitoyl*** lysines (PL) with alternating chirality. An 18-residue peptide from the third variable region in the gp120 of HIV-1 was used as antigen in eight models for a structure-function study. Alternating ***palmitoyl*** lysine (PL) was introduced as the lipid anchor and built-in adjuvant because D and L Lys (Pal) was found via molecular modeling to best mimic phosphatidylcholine and thus provide the most stable peptide antigens on the ordered lipid membranes. The requirements of the ***palmitoyl*** lysines and the L-Ser-L-Ser linker were crucial, since replacement with ***palmitoyl*** serines or L-Ser-D-Ser linkers led to a marked decrease in immune response. The stoichimetric ratio of PL vs MAP was also important. Multiple antigen peptide (MAP) constructs without the lipophilic PLs, those that were underlipidated and contained one PL, or those that were overlipidated containing four PLs, were ineffective. LipoMAPs containing three palmitic acids elicited significant humoral responses in oil-based emulsion and liposomes, but not in water or alum formulations. LipoMAP containing only two PLs was found best to be incorporated in liposomes and elicited a significant immune response and cytotoxic T-lymphocytes (CTLs). These models were compared favorably with a preparation using tripalmitoyl-S-glyceryl cysteine (P3C) as the lipid anchor. We also developed a modular synthesis of MAP-P3C that incorporated P3C as a premade unit containing a thiopyridine, which simplified the overall scheme and minimized oxidation during stepwise peptide synthesis. This lipoMAP model is a new addition to the design of our macromolecular assemblage approach mimicking peptide antigens on the surface of micro-organisms. It may be a potentially useful approach to the design of a synthetic ***vaccine*** for humans.

L24 ANSWER 26 OF 39 MEDLINE
95156422 Document Number: 95156422. PubMed ID: 7853339. Pimelautide or trimexautide as built-in adjuvants associated with an HIV-1-derived peptide: synthesis and in vivo induction of antibody and virus-specific cytotoxic T-lymphocyte-mediated response. Deprez B; Gras-Masse H; Martinon F; Gomard E; Levy J P; Tartar A. (Faculte de Pharmacie, URA CNRS 1309, Institut Pasteur de Lille, France.) JOURNAL OF MEDICINAL CHEMISTRY, (1995 Feb 3) 38 (3) 459-65. Journal code: J0F; 9716531. ISSN: 0022-2623. Pub. country: United States. Language: English.

AB Covalent association of lipopeptidic immunostimulants is known to improve the immunogenicity of short peptides. In this paper, we describe the synthesis of four analytically pure immunogens, prepared by two different strategies, in which a hexadecameric peptide (V3) derived from the principal neutralizing domain of HIV-1 envelope glycoprotein was associated with two different murein-derived ***lauroyl*** -peptides, Pimelautide (RP 44102), or Trimexautide (RP 56142). The in vivo immunogenicity of these compounds was evaluated according to two different criteria: the ability to elicit a cellular-T cytotoxic (CTL response) and the ability to stimulate antibody response. Our studies show that one of our compounds (TrxSucV3) was able to efficiently induce a relevant virus-specific CTL response, while another one (PimSucV3) was able to stimulate a strong antibody response to the linked peptide, or to a co-injected protein. These results suggest that both activities rely on different structure-activity relationships and that such a chemically defined model of peptide ***vaccines*** may be used to selectively

stimulate subpopulations of immunocompetent cells.

L24 ANSWER 13 OF 39 MEDLINE
1998065482 Document Number: 98065482. PubMed ID: 9401920. Synthetic peptide ***vaccines*** : palmitoylation of peptide antigens by a thioester bond increases immunogenicity. Beekman N J; Schaaper W M; Tesser G I; Dalsgaard K; Kamstrup S; Langeveld J P; Boshuizen R S; Meloen R H. (Institute for Animal Science and Health (ID-DLO), Lelystad, The Netherlands.) JOURNAL OF PEPTIDE RESEARCH, (1997 Nov) 50 (5) 357-64. Journal code: CTZ; 9707067. ISSN: 1397-002X. Pub. country: Denmark. Language: English.

AB Synthetic peptides have frequently been used to immunize animals. However, peptides less than about 20 to 30 amino acids long are poor immunogens. In general, to increase its immunogenicity, the presentation of the peptide should be improved, and molecular weight needs to be increased. Many attempts have been made to couple peptide immunogens to different carrier proteins [e.g. keyhole limpet haemocyanin (KLH) or ovalbumin]. This leads to very complex structures, however. We used a controlled conjugation of a peptide to a single long-chain fatty acid like palmitic acid by a thioester or an amide bond. It was found that these S-***palmitoylated*** peptides were much more immunogenic than N-***palmitoylated*** peptides and at least similar to KLH-conjugated peptides with respect to appearance and magnitude of induced antibodies (canine parvovirus) or immunocastration effect (gonadotropin-releasing hormone). For chemical synthesis of thioesters, we established conditions for solution and solid-phase synthesis. In both phases, Cys(SBut) could only be deprotected efficiently using phosphines, and S-acylation was accomplished using standard coupling at pH 5. We speculate that, in vivo, the presence of an appropriate fatty acid chain, chemically linked through a labile thioester bond, greatly enhances immunogenicity, because it represents a favourable substrate for cleavage by cellular thioesterases in cells of the immune system.

L24 ANSWER 18 OF 39 MEDLINE
97158176 Document Number: 97158176. PubMed ID: 9004448. Synthetic ***vaccine*** against foot-and-mouth disease based on a ***palmitoyl*** derivative of the VP1 protein 135-159 fragment of the A22 virus strain. Volpina O M; Yarov A V; Zhmak M N; Kuprianova M A; Chepurkin A V; Toloknov A S; Ivanov V T. (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya, Moscow, Russian Federation.) VACCINE, (1996 Oct) 14 (14) 1375-80. Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The peptide Palm2 135-159, a dipalmitoyl derivative of the 135-159 fragment of VP1 protein of the foot-and-mouth disease virus strain A22 was synthesized. In the experiments on mice, guinea pigs and sheep Palm2 135-159 possesses greater immunogenic and protective activity than the nonacylated 135-159 peptide. The synthetic ***vaccine*** against foot-and-mouth disease for use in sheep was developed on the basis of the lipopeptide. Synthetic polymethylsiloxane oil was found to be a suitable adjuvant for this ***vaccine*** . The dependencies of protective and immunogenic effects from the dose of peptide were studied. The ***vaccine*** was found to be stable to storage for 1 year at 18 degrees C. It was shown that the synthetic ***vaccine*** provides 1 year protection of sheep against foot-and-mouth disease after a single administration. The ***vaccine*** is allowed for veterinary use in Russia.

L24 ANSWER 21 OF 39 MEDLINE

96363706 Document Number: 96363706. PubMed ID: 8719518. Priming for virus-specific CD8+ but not CD4+ cytotoxic T lymphocytes with synthetic lipopeptide is influenced by acylation units and liposome encapsulation. Babu J S; Nair S; Kanda P; Rouse B T. (Department of Microbiology, College of Veterinary Medicine, University of Tennessee, Knoxville 37996-0845, USA.) VACCINE, (1995 Dec) 13 (17) 1669-76. Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Synthetic peptides of the herpes simplex virus glycoprotein B synthesized either as a free form or derivatized with one (PAM1) or three palmitic acids (PAM3Cys) were used to assess the in vivo priming efficacy of high affinity virus-specific CTL induction. The peptide and its derivatives were delivered in vivo with or without liposome encapsulation. Neither the free peptide nor the PAM1 derivative primed for high affinity virus specific CD8+ CTL induction, whether delivered via liposomes or not. On the other hand, the PAM3Cys derivative was able to prime for low levels of high affinity virus specific CD8+ CTL induction in the absence of liposome encapsulation. However, the efficiency of virus-specific CD8+ CTL induction with PAM3Cys derivative was enhanced following encapsulation in the liposomes. In contrast, all forms of the peptides induced both CD4+ T cell proliferative response as well as high affinity virus-specific CD4+ CTL. In addition, the efficiency of the PAM3Cys derivative to prime for CD4+ or CD8+ CTL was found to be influenced by the liposome encapsulation. When delivered via liposomes, the PAM3Cys derivative effectively primed for CD8+ CTL. However, liposomal delivery was not necessary for efficient priming for CD4+ CTL induction. Thus, both the acylation units as well as liposomal delivery appear to influence the in vivo priming of CD4+ and CD8+ T cell responses with synthetic peptides.

L24 ANSWER 26 OF 39 MEDLINE

95156422 Document Number: 95156422. PubMed ID: 7853339. Pimelautide or trimexautide as built-in adjuvants associated with an HIV-1-derived peptide: synthesis and in vivo induction of antibody and virus-specific cytotoxic T-lymphocyte-mediated response. Deprez B; Gras-Masse H; Martinon F; Gomard E; Levy J P; Tartar A. (Faculte de Pharmacie, URA CNRS 1309, Institut Pasteur de Lille, France.) JOURNAL OF MEDICINAL CHEMISTRY, (1995 Feb 3) 38 (3) 459-65. Journal code: J0F; 9716531. ISSN: 0022-2623. Pub. country: United States. Language: English.

AB Covalent association of lipopeptidic immunostimulants is known to improve the immunogenicity of short peptides. In this paper, we describe the synthesis of four analytically pure immunogens, prepared by two different strategies, in which a hexadecameric peptide (V3) derived from the principal neutralizing domain of HIV-1 envelope glycoprotein was associated with two different murein-derived ***lauroyl*** -peptides, Pimelautide (RP 44102), or Trimexautide (RP 56142). The in vivo immunogenicity of these compounds was evaluated according to two different criteria: the ability to elicit a cellular-T cytotoxic (CTL response) and the ability to stimulate antibody response. Our studies show that one of our compounds (TrxSucV3) was able to efficiently induce a relevant virus-specific CTL response, while another one (PimSucV3) was able to stimulate a strong antibody response to the linked peptide, or to a co-injected protein. These results suggest that both activities rely on different structure-activity relationships and that such a chemically defined model of peptide ***vaccines*** may be used to selectively stimulate subpopulations of immunocompetent cells.

L6 ANSWER 1 OF 4 USPATFULL
1999:145984 Oral or intranasal vaccines using hydrophobic complexes having proteosomes and lipopolysaccharides.
Lowell, George H. , 6303 Western Run Dr., Baltimore, MD, United States 21215
US 5985284 19991116
APPLICATION: US 1996-677302 19960709 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An immunogenic complex, essentially consisting of neisserial outer membrane protein proteosomes hydrophobically complexed to native purified bacterial lipopolysaccharide and formulated in accordance with the current invention for mucosal delivery such as via the oral or intranasal route is used as a vaccine. Specifically, a vaccine using shigella lipopolysaccharides complexed to proteosomes for such mucosal administration induces IgG and IgA antibodies in sera and in respiratory and intestinal fluids. Furthermore, such antibodies are associated with protection against shigella infection and these vaccines are herein demonstrated to protect against mucosal infection with shigella.

CLM What is claimed is:
1. An immunogenic hydrophobic complex consisting essentially of proteosomes and at least one non-detoxified antigenic lipopolysaccharide.

2. The immunogenic hydrophobic complex of claim 1 wherein the lipopolysaccharide is isolated from Shigella.

3. The immunogenic hydrophobic complex of claim 2 wherein the Shigella is selected from the group consisting of S. flexneri 2a and Shigella sonnei or mixtures thereof.

4. The immunogenic hydrophobic complex of claim 1 wherein the proteosomes are derived from N. meningiditis.

5. The immunogenic hydrophobic complex of claim 1 wherein the proteosomes are derived from N. gonorrhea.

6. A vaccine comprising the immunogenic hydrophobic complex according to any one of claims 1, 2, 3, 4 and 5 and a carrier.

7. A method for providing enhanced immunogenicity comprising administering the vaccine of claim 6 to a subject parenterally, orally, intranasally or topically.

8. A method of achieving immunity by administering the vaccine of claim 6 to a subject parenterally, orally, intranasally or topically to impart immunity.

9. A method of achieving immunity according to claim 8 wherein the immunity is to gram negative bacterial infection.

10. A method of achieving immunity according to claim 9 wherein the immunity is to neisserial infection.

11. A method of achieving immunity according to claim 10 wherein the immunity is to gonococcal infection.

12. A method of achieving immunity according to claim 10 wherein the

immunity is to meningococcal infection.

13. A method of achieving immunity according to claim 8 wherein the immunity is to shigellosis.

14. A method of achieving immunity according to claim 13 wherein the shigellosis immunity is to Shigella flexneri 2a.

15. A method of achieving immunity according to claim 13 wherein the shigellosis immunity is to Shigella sonnei.

16. A method of achieving immunity according to claim 8 by administering the vaccine to mucosal surfaces selected from the group of respiratory, gastrointestinal, vaginal, nasal, rectal and oral mucosa.

L6 ANSWER 2 OF 4 USPATFULL
1999:120874 Submicron emulsions as vaccine adjuvants.
Lowell, George H. , Baltimore, MD, United States
Amselem, Shimon, Rehovot, Israel
Friedman, Doron, Carmei Yosef, Israel
Aviv, Haim, Rehovot, Israel
Pharmos Corporation, New York, NY, United States (U.S. corporation)The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 5961970 19991005
WO 9511700 19950504
APPLICATION: US 1996-637756 19960429 (8)
WO 1993-US10402 19931029 19960429 PCT 371 date 19960429 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A vaccine adjuvant composition of an oil-in-water submicron emulsion that has about 0.5 to 50% of a first component of an oil, about 0.1 to 10% of a second component of an emulsifier, about 0.05 to 5% of a nonionic surfactant, about 0.00001 to 1% of an immunogen, and an aqueous continuous phase. This submicron emulsion has a mean droplet size in the range of between about 0.03 and 0.5 .mu.m, and preferably 0.05 and 0.2 .mu.m.

CLM What is claimed is:
1. A vaccine adjuvant composition of an oil-in-water submicron emulsion consisting essentially of about 0.5 to 50% of a first component of an oil, about 0.1 to 10% of a second component of an emulsifier, wherein the emulsifier is a phospholipid compound or a mixture of phospholipids selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin, about 0.05 to 5% of a non-ionic surfactant, about 0.00001, to 1% of an immunogen, and an aqueous continuous phase, said submicron emulsion having a mean droplet size in the range of between about 0.03 and 0.5 .mu.m, which composition is substantially free of added amounts of muramyl peptides or their lipophilic derivatives.

2. The emulsion of claim 1 wherein said mean droplet size is between about 0.05 and 0.2 .mu.m.

3. The emulsion of claim 1, wherein said first component is selected from the group consisting of a medium chain triglyceride oil, a vegetable oil and mixtures thereof.

4. The emulsion of claim 3 wherein said first component is present in an

amount of about 1 to 20%.

5. The emulsion of claim 1 wherein said emulsifier is present in an amount of about 0.2 to 5%.

6. The emulsion of claim 1 wherein said surfactant is selected from the group consisting of fatty alcohols, polyethylene glycol esters of fatty acids, polyethoxylated fatty acid esters, polyethoxylated fatty alcohol ethers and polyethoxylated alkylphenyl ethers.

7. The emulsion of claim 1 wherein said surfactant is an alkylene oxide condensate of an organic compound having one or more hydroxyl groups.

8. The emulsion of claim 7 wherein said non-ionic surfactant is present in an amount of about 0.1 to 5%.

9. The emulsion of claim 1 wherein said first component is present in an amount of about 1 to 20%, and the second component and the non-ionic surfactant are each present in an amount of about 0.1 to 2%.

10. The composition of claim 1 wherein said immunogen is selected from the group consisting of hydrophilic, lipophilic and amphiphilic immunogens.

11. The composition of claim 1 wherein said immunogen is selected from the group consisting of native, recombinant and synthetic peptides, proteins and glycoproteins derived from a source selected from the group consisting of bacteria, viruses and parasites.

12. The composition of claim 11 wherein said immunogen is the gp160 envelope protein of the HIV virus, or a fragment thereof.

13. The composition of claim 11 wherein said immunogen is the surface glycoprotein of a Leishmania parasite or a fragment thereof.

14. The composition of claim 13 wherein said surface glycoprotein or peptide is covalently conjugated to a hydrophobic foot.

15. The composition of claim 14 wherein said hydrophobic foot is lauryl-cysteine.

16. The composition of claim 1 wherein said immunogen is a protein toxoid.

17. The composition of claim 16 wherein said immunogen is Staphylococcus Enterotoxin B toxoid.

18. The composition of claim 1 wherein said immunogen is selected from the group consisting of an oligosaccharide, a polysaccharide and a lipopolysaccharide.

19. The composition of claim 18 wherein said immunogen is a lipopolysaccharide from Shigella flexneri.

20. The composition of claim 1 wherein said immunogen is completed to proteosomes.

21. A method for preparing the composition of claim 1 by an intrinsic procedure, which comprises adding the immunogen to the oil phase before emulsification of the water and oil phases.

22. A method for preparing the composition of claim 1 by an intrinsic procedure, which comprises adding the immunogen to the water phase before emulsification of water and oil phases.

23. A method for preparing the composition of claim 1 by an extrinsic procedure, which comprises preparing a submicron emulsion from the oil, surfactant and emulsifier components, and adding the immunogen externally by mixing it with the previously prepared submicron emulsion.

24. A method for providing enhanced immunogenicity in a subject which comprises administering the composition of claim 1, to the subject to introduce the immunogen therein.

25. The method of claim 24 which further comprises administering the composition parenterally, orally, intranasally or topically to the subject.

L6 ANSWER 3 OF 4 USPATFULL
1998:25345 Immuno-potentiating systems for preparation of immunogenic materials

Lowell, George H. , 6303 Westlin Run Dr., Baltimore, MD, United States 21215
US 5726292 19980310
APPLICATION: US 1993-143365 19931029 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention is directed to improved immunopotentiating systems for preparation of immunogenic materials. More particularly, the invention is directed to immunogenic compositions containing a protein, polypeptide, or peptide, a hydrophobic anchor, and a proteosome. The immunogenic compositions are suitable for use as therapeutic agents and vaccines.

CLM What is claimed is:
1. A construct comprising: (1) a protein, protein fragment, polypeptide or peptide, (2) a hydrophobic anchor, and (3) a proteosome.

2. A construct of claim 1 wherein the hydrophobic anchor is attached to the protein, polypeptide, or peptide through one or more cysteines.

3. A construct of claim 2 wherein a disulfide bond exists between two cysteine residues.

4. A construct of claim 1 wherein the hydrophobic anchor is an alkanoyl moiety.

5. A construct of claim 1 wherein the hydrophobic anchor is a lauroyl moiety.

6. A construct of claim 5 wherein the hydrophobic anchor is a peptide.

7. A construct of claim 1 containing the peptide Gly Asn Val Gln Ala Ala Lys Asp Gly Gly Asn Thr Ala Ala Gly Arg (Seq. No. 15).

8. A construct of claim 1 wherein the hydrophobic anchor is a lauryl moiety bound to the peptide through a Cysteine.

9. A method of making a construct of claim 1 comprising the steps of: (1) replicating a core peptide or protein; (2) reacting said replicated

peptide or protein with an aliphatic carboxylic acid or a hydrophobic peptide to add a hydrophobic anchor to said peptide or protein; and (3) complexing the structure formed in step (2) with a proteosome.

10. A method of claim 9 wherein, after step (1) and before step (2), the peptide or protein is reacted with cysteine to add at least one cysteine residue to said replicated peptide or protein.

11. A method of step 9 wherein the product of step (2) is complexed with the proteosome by dialysis.

12. A method of claim 9 wherein the product of step (2) is complexed to the proteosome by lyophilization.

13. A construct of claim 1 containing the peptide Phe Leu Leu Ala Val (Seq. No. 1).

14. A construct of claim 1 containing the Leishmania gp63 peptide.

15. A construct comprising: (1) a protein, polypeptide or peptide, (2) a hydrophobic anchor, said anchor being a peptide of formula Gly Gly Tyr Cys Phe Val Ala Leu Leu Phe (SEQ ID NO: 11), and (3) a proteosome.

L6 ANSWER 4 OF 4 USPATFULL
1998:14497 Solid fat nanoemulsions as vaccine delivery vehicles.
Anselem, Shimon, Rehovot, Israel
Lowell, George H. , Baltimore, MD, United States
Aviv, Haim, Rehovot, Israel
Friedman, Doron, Carmei Yosef, Israel
Pharmos Corporation, New York, NY, United States (U.S. corporation)The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 5716637 19980210
WO 9426255 19941124
APPLICATION: US 1995-553350 19951116 (8)
WO 1994-US5589 19940518 19951116 PCT 371 date 19951116 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides pharmaceutical vaccine compositions that are nanoemulsions of particles having a lipid core which is in a solid or liquid crystalline phase at 25.degree. C., and which is surrounded by at least one phospholipid bilayer for the parenteral, oral, intranasal, rectal, vaginal or topical delivery of both hydrophilic and lipophilic immunogens. The particles have a mean diameter in the range of 10 to 250 nm and the immunogen is incorporated therein, either intrinsically prior to the homogenization process or extrinsically thereafter.

CLM What is claimed is:
1. A pharmaceutical composition for the administration of antigen which comprises a nanoemulsion of a plurality of noncellular lipid particles having a mean diameter of about 10 to 250 nm, as determined on a weight basis, the particles being suspended in an aqueous continuous phase, wherein each said lipid particle comprises a lipid core composed of a lipid which is a solid or liquid crystal as determined in bulk at a temperature of about 25.degree. C. or higher, and at least one phospholipid bilayer surrounding said core and encapsulating a portion of said aqueous continuous phase, said particles entrapping about 0.001 to 5% of an immunogen in said lipid core or in said encapsulated aqueous phase.

2. The pharmaceutical composition of claim 1 wherein the mean particle diameter of said lipid particles falls within the range of about 20 to 180 nm as determined on a weight basis.

3. The pharmaceutical composition of claim 2 wherein the particle diameter of at least 99% of said lipid particles falls within the range of about 50 to 150 nm as determined on a weight basis.

4. The pharmaceutical composition of claim 2 wherein the lipid core comprises a fatty acid ester.

5. The pharmaceutical composition of claim 4 wherein the lipid core has a solid to fluid phase transition temperature below 37.degree. C. as determined in bulk.

6. The pharmaceutical composition of claim 4 wherein the lipid core comprises a triglyceride.

7. The pharmaceutical composition of claim 6 wherein said triglyceride comprises a fatty acid moiety of C.sub.10 to C.sub.18.

8. The pharmaceutical composition of claim 6 wherein said triglyceride is completely saturated.

9. The pharmaceutical composition of claim 6 wherein said triglyceride is selected from the group consisting of tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin.

10. The pharmaceutical composition of claim 6 wherein the mole ratio of phospholipid to total lipid is in the range of from 0.1:1 to 0.5:1.

11. The pharmaceutical composition of claim 6 wherein the weight ratio of phospholipid to triglyceride is in the range of from 0.5:1 to 1.5:1.

12. The pharmaceutical composition of claim 4 wherein said phospholipid is a phosphatidylcholine.

13. The pharmaceutical composition of claim 12 wherein said phosphatidylcholine is egg phosphatidylcholine.

14. The pharmaceutical composition of claim 12 wherein said phosphatidylcholine has a transition temperature below 25.degree. C.

15. The pharmaceutical composition of claim 12 wherein said phosphatidylcholine is saturated.

16. The pharmaceutical composition of claim 1 wherein said lipid particle contains cholesterol or cholesteryl esters.

17. The composition of claim 1 wherein the immunogen is hydrophilic, lipophilic, or amphophilic.

18. The composition of claim 1 wherein the immunogen is a peptide, protein, or glycoprotein.

19. The composition of claim 18 wherein the antigen is the gp160 envelope protein of the HIV virus, or a fragment thereof.

20. The composition of claim 18 wherein the antigen is the surface glycoprotein of a Leishmania parasite, or a fragment thereof.

21. The composition of claim 20 wherein the surface glycoprotein or peptide is covalently conjugated to a hydrophobic component.

22. The composition of claim 21 wherein the hydrophobic component is lauryl-cysteine.

23. The composition of claim 1 wherein the immunogen is a protein toxoid.

24. The composition of claim 23 wherein the immunogen is Staphylococcus Enterotoxin B toxoid.

25. The composition of claim 1 wherein the immunogen is complexed with a proteosome.

26. The composition of claim 1 wherein the nanoemulsion further comprises a bioadhesive or mucoadhesive macromolecule.

27. The composition of claim 26 wherein the said mucoadhesive macromolecule is a polymer.

28. The composition of claim 26 wherein the said mucoadhesive macromolecule is selected from the group of acidic nonnatural polymers consisting of polymers and copolymers containing acrylic acid units, polymers and copolymers containing methacrylic acid units, and poly(methylvinylether/maleic anhydride) copolymer.

29. The composition of claim 28 wherein the said polymer is polyacrylic acid.

30. The composition of claim 1 which contains no added muramyl peptides.

31. The pharmaceutical composition of claim 1 wherein said lipid particle is substantially free of lipase and phospholipase activity.

32. A method for delivery of an immunogen to an animal, comprising administering to said animal a pharmaceutical according to claim 1.

33. The method of claim 32 wherein the mean diameter of the lipid particles in said composition is in the range of about 20 to 180 nm.

34. The method of claim 32 wherein said composition is administered parenterally, orally, intranasally, or topically, thereby providing enhanced immunogenicity.

35. The method of claim 32 wherein said composition is administered to mucosal surfaces, thereby achieving mucosal immunity.

36. A method for making a nanoemulsion for administration of an immunogen, comprising the steps of: preparing a mixture comprising phospholipid and triglyceride in the weight ratio range of about 0.5:1 to 1.5:1 wherein said triglyceride has a solid to liquid phase transition temperature of greater than 25.degree. C.; suspending said mixture in an aqueous solution at a temperature below the solid to liquid transition temperature of the triglyceride; reducing the size of the suspension to yield a nanoemulsion of lipid particles having a mean particle diameter of between about 10 nm and 250 nm; and incorporating an immunogen in the nanoemulsion.

37. The method according to claim 36 for preparing the composition of

the nanoemulsion by an intrinsic procedure, where the immunogen is added before homogenization of water and oil phases.

38. The method of claim 36 for preparing the composition of the nanoemulsion by an extrinsic procedure, where the immunogen is added externally by mixing with the previously prepared plain nanoemulsion.

39. A pharmaceutical composition comprising dehydrated lipid particles containing an antigen for administration as a nanoemulsion, wherein said lipid particles comprise a lipid core surrounded by at least one phospholipid layer, said lipid core is composed of lipid in a solid or liquid crystalline phase at least about 25.degree. C. as determined in bulk, and said lipid particles have a mean diameter upon rehydration of about 10 to 250 nm.

40. The pharmaceutical composition of claim 39 further comprising a cryoprotectant.

41. The pharmaceutical composition of claim 40 wherein said cryoprotectant is selected from the group consisting of glucose, sucrose, lactose, maltose, trehalose, dextran, dextrin, cyclodextrin, polyvinylpyrrolidone, and amino acids.

42. The pharmaceutical composition of claim 40 wherein said cryoprotectant is present in a range of from 0.1% to 10% by weight compared to lipid.

43. The pharmaceutical composition of claim 39 wherein said lipid particles contain an immunogen.

44. A method for delivering an antigen to an animal comprising administering to said animal a pharmaceutical composition according to claim 39.

L36 ANSWER 10 OF 12 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1994-048884 [06] WPIDS
DNC C1994-022172
TI New conjugates for treating or preventing ***HIV*** infection - comprising ***HIV*** -specific neutralisation epitopes covalently linked to outer membrane ***proteosome*** of Neisseria.
DC B04 D16
IN ARNOLD, B A; CONLEY, A J; KELLER, P M; SHAW, A R
PA (MERI) MERCK & CO INC
CYC 42
PI WO 9402626 A1 19940203 (199406)* 180p
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE
W: AU BB BG BR BY CA CZ FI HU JP KR KZ LK MG MN MW NO NZ PL RO RU SD SK UA US
AU 9346830 A 19940214 (199425)
ADT WO 9402626 A1 WO 1993-US6751 19930719; AU 9346830 A AU 1993-46830 19930719, WO 1993-US6751 19930719
FDT AU 9346830 A Based on WO 9402626
PRAI US 1992-917212 19920720; US 1992-917214 19920720; US 1992-917215 19920720; US 1992-917217 19920720

AB WO 9402626 A UPAB: 19940322
An antigenic conjugates of ***HIV*** -specific, selected principal neutralisation epitopes covalently linked to purified outer membrane ***proteosome*** of Neisseria and its salts are new, where the conjugate is of formula (I) (SPNE)n-(OMPC) (I) SPNE is the selected principal neutralisation epitope of ***HIV*** , which is a polypeptide of one or

more amino acid sequences shown or their fragments, the fragments having at least 5 amino acids and including the GPXR loop region or homologues; n indicates the number of polypeptides of SPNE covalently linked to OMPC and is 1-50; - indicates a covalent linkage; OMPC is purified outer membrane ***proteosome*** of Neisseria, the conjugate being opt. substd. with a-, which is an anion or polyanion at physiological pH, the a- consisting of 1-5 residues of anions selected from carboxylic, sulphonic, propionic or phosphonic acid.

USE - The SPNEs are useful as neutralisation epitopes specific for ***HIV*** . Their conjugates with OMPC are useful for ***vaccination*** against AIDS or ARC and for prodn. of ***HIV*** -specific broadly neutralising antibodies for passive immunity against AIDS or ARC. The conjugates can also be used for treating or preventing ***HIV*** infection. The SPNEs and their conjugates are also useful in the detection of ***HIV*** or antibodies to ***HIV*** , for screening, clinical evaluation and diagnosis. The phage epitope screening method provides for the efficient identification of antigenic determinants.